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PCT/EP 03/04650
Applicant/Proprietor:
DeveloGen Aktiengesellschaft für entwicklungsbiologische Forschung

In response to the official communication dated June 26, 2003.

Encl.
Sequence Listing (diskette) ✓
Sequence Listing (paper copy) (all 1-fold) ✓
New description pages 60 ✓ and 61 ✓

Applicants herewith submit a sequence listing in computer-readable form as well as in the form of a paper copy.

It is stated that the information recorded on the data carrier is identical to the written sequence listing. It is further stated that the sequence listing does not include subject matter which goes beyond the content of the application as originally filed.

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Furthermore, new description pages 60 and 61 are filed including the correct sequence numberings.

Dr. W. Weiss

temperature (preferrably 22°C), 40 per cent humidity and a light / dark cycle of preferrably 14 / 10 hours. The mice were fed a standard chow (for example, from ssniff Spezialitäten GmbH, order number ssniff M-Z V1126-000). For the fasting experiment ("fasted wild type mice"), wild type mice were starved for 48 h without food, but only water supplied ad libitum (see, for example, Schnetzler et al., (1993) J Clin Invest 92(1):272-280, Mizuno et al., (1996) Proc Natl Acad Sci U S A 93(8):3434-3438). Animals were sacrificed at an age of 6 to 8 weeks. The animal tissues were isolated according to standard procedures known to those skilled in the art, snap frozen in liquid nitrogen and stored at -80°C until needed.

RNA was isolated from mouse tissues using Trizol Reagent (for example, from Invitrogen, Karlsruhe, Germany) and further purified with the RNeasy Kit (for example, from Qiagen, Germany) in combination with an DNase-treatment according to the instructions of the manufacturers and as known to those skilled in the art. Total RNA was reverse transcribed (preferrably using Superscript II RNaseH- Reverse Transcriptase, from Invitrogen, Karlsruhe, Germany) and subjected to Taqman analysis preferrably using the Taqman 2xPCR Master Mix (from Applied Biosystems, Weiterstadt, Germany; the Mix contains according to the Manufacturer for example AmpliTaq Gold DNA Polymerase, AmpErase UNG, dNTPs with dUTP, passive reference Rox and optimized buffer components) on a GeneAmp 5700 Sequence Detection System (from Applied Biosystems, Weiterstadt, Germany).

Taqman analysis was performed preferrably using the following primer/probe pairs:

For the amplification of Sac domain-containing inositol phosphatase 2 (sac2) (SEQ ID NO:2): 5'- CCT GGA TCG CAC CAA CG -3'; mouse sac2 reverse primer (SEQ ID NO:3): 5'- TTA AGC TGC TGT TCC ATG ACC A

-3'; Taqman probe (SEQ ID NO:4): (5/6-FAM) TCC AGG CTG CCA TAG CGC GC (5/6-TAMRA)

For the amplification of mouse solute carrier family 25 (mitochondrial carrier, Aralar) member 12 (Slc25a12) (SEQ ID NO:5): 5'- CCT GCC AAC CCT GAT CAC A -3'; mouse Slc25a12 reverse primer (SEQ ID NO:6): 5'- TTT CAA TGC CAG CGA AAG TG -3'; Taqman probe (SEQ ID NO:7): (5/6-FAM) CGG TGG CTA CAG ACT TGC CAC GG (5/6-TAMRA)

For the amplification of mouse solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 13 (Slc25a13) (SEQ ID NO: 8): 5'- AGC GGT GGT TCT ATG TCG ATT T -3'; mouse Slc25a13 reverse primer (SEQ ID NO: 9): 5'- CGG GAT TTA GGA ACC GGC T -3'; Taqman probe (SEQ ID NO:10): (5/6-FAM) AGG CGT GAA GCC CGT GGG ATC T (5/6-TAMRA)

For the amplification of mouse myelin gene expression factor 2 (mef2) (SEQ ID NO: 11): 5'- ACA AGG ATG GCA AGA GCA GAG -3'; mouse mef2 reverse primer (SEQ ID NO: 12): 5'- ATG GAA ATT GCT TGG ACT GCT T -3'; Taqman probe (SEQ ID NO:13): (5/6-FAM) CAT GGG CAC TGT CAC TTT TGA GCA GG (5/6-TAMRA)

In the figures the relative RNA-expression is shown on the Y-axis. In Figures 4A and B, 8A, B, C, and D, and 16A, B, and C, the tissues tested are given on the X-axis. "WAT" refers to white adipose tissue, "BAT" refers to brown adipose tissue.

As shown in Figure 4A, real time PCR (Taqman) analysis of the expression of the Sac domain-containing inositol phosphatase 2 (SAC2) RNA in mammalian (mouse) tissues revealed that SAC2 is highly expressed in hypothalamus, brain, WAT, spleen and kidney. Figure 4B shows that SAC2 is upregulated in BAT and pancreas of fasted animals as well as ob / ob